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Prevalence of *Wolbachia* infection in mosquito populations in Sri Lanka

N.D.A.D. Wijegunawardana[1*], **Y.I.N. Silva Gunawardene**[1],
T.G.A.N. Chandrasena[2], **R.S. Dassanayake**[3], **P.A.D.H.N. Gunathilaka**[2],
P. Kittayapong[4], **Z. Xi**[5], **K. Bourtzis**[6] **and W. Abeyewickreme**[1, 2]

[1]Molecular Medicine Unit, [2]Department of Parasitology, Faculty of Medicine, University of Kelaniya, Sri Lanka, [3]Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka, [4]Center of Excellence for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Thailand, [5]Sun Yat-Sen University - Michigan State University Joint Center of Vector Control for Tropical Diseases, China, [6]Joint FAO/IAEA Insect Pest Control Laboratory, Seibersdorf, Austria
**asha@kln.ac.lk*

During the last ten years, intensive research efforts have provided evidence that insect symbionts like *Wolbachia* can be useful tools for the control of major agricultural pests and disease vectors, including *Aedes* mosquitoes. The objective of the present study was to determine and characterize the presence of *Wolbachia* infections in wild mosquito populations in Sri Lanka in order to identify suitable strains which may be useful in the combined SIT / IIT approach. Adult mosquitoes were collected from 15 selected districts in Sri Lanka namely; Gampaha, Colombo, Galle, Matara, Hambanthota, Jaffna, Mannar, Ampara, Trincomalee, Batticaloa, Anuradhapura, Kandy, Kegalle, Badulla and Nuwara Eliya from October, 2014 to March, 2016. Mosquitoes were identified into the species leveland stored in -20 ^{0}C freezer after labeling until taken for molecular analysis. The field caught mosquito specimens were processed for the genomic DNA extraction individually using Qiagenkits (Qiagen DY14, Hilden, Germany). The DNA amplification was carried out using a specific pair of primersthat recognizes the 16S *rRNA* gene of *Wolbachia*. Primers which amplify the gene encoding for the major *Wolbachia* surface protein (*wsp*) were also used for screening in order to confirm the results. Two negative controls (*Wolbachia* un-infected mosquito DNA and milli-Q water) and one positive control (*Wolbachia*double infected *Aedes albopictus* Thailand strain) were used at each Polymerase Chain Reaction (PCR). Each PCR product of 5µl was subjected to gel electrophoresis and visualized in gel image system. The results were compared with the marker (100bp ladder) in an attempt to identify band sizes of about 1 kb and 540-632bp for the 16S *rRNA* and *wsp*genes, respectively. The experiment was conducted for 3, 24, 2, 5, 16, 7 and 21 species from each mosquito genus *Tripteroides, Anopheles*, *Toxorhynchites*, *Mansonia*, *Aedes*, *Armigerus* and *Culex* respectively. For confirmation of the results, each PCR reaction was repeated three times.PCR amplicons were further analyzed by nucleotide sequencing, Blastn search and sequence alignment. Accordingly, a total of 78 mosquito species were screened and twelve (12) of them namely; *Mansoniaindiana*, *Mn. uniformis*, *Mn. annulifera, Aedesalbopictus*, *Ae. pseudalbopictus*, *Armigeressubalbatus*, *Ar. flavus*, *Culexgelides, Cx. quinquefaciatus*, *Cx.sinensis*and*Cx.sitiens*were positive by both PCR assays. None of the *Anophele*s species or *Aedesaegypti* was found infected with *Wolbachia*. Sequence analysis indicated significant genetic diversity among the various *Wolbachia*strains detected.

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